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Construction of Effective Microorganism (EM) Like Microbial Consortia and Their Growth Promoting Effect on *Arachis hypogea* (L.).

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ABSTRACT

In the present study, construction of Effective Microorganisms like microbial consortia and the growth promoting effect on *Arachis hypogeal* has been carried out. The experiment was performed in micro plot representing various conditions in the field. It consists of three treatments replicated five times arranged in a complete randomized design. One set of treatment consist of untreated control, while the other treatment consist of EM like microbial consortia constructed in our laboratory and standard EM formulation. Shoot length, surface area of leaf, height of the plant, total new branches emerged per plant, total foliage leaves per plant, total heterotrophic microbial population from phyllosphere, total microbial population from soil, chlorophyll estimation, pest infestation, diseased spots and healthy yield of pod was found to be significantly increased in EM like microbial consortia treatment followed by standard. There was no significant difference in leaf chlorophyll content among all the treatments. Moreover the nitrogen, phosphorous and potassium level was found to be increased in EM like microbial consortia treated plots. The total heterotrophic microbial population in soil and phyllosphere was also increased. Yield and cost benefit ratio was also high in test treatment. The results from the study demonstrated that growth and yield of groundnut improved by inoculating the plant with EM like microbial consortia and as a result reduce the use of fertilizers in production of groundnut, hence promoting sustainable agriculture.

Keywords: effective microorganism, *Arachis hypogea,* microbial consortia, growth parameters

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INTRODUCTION

Peanut is the one of the most important edible oil crops which due to its high nutritive value of its seeds for human and the produced cake as well as the green leaf hay for livestock, in addition to the importance seed oil for industrial purposes [1]. Increasing of peanut production in order to cover the local consumption and exported outside could be achieved by introducing high productivity varieties and improving the cultural practices and managements as well as chosen the proper planting density- peanut crop has different groups of varieties [2].Use of soil microorganisms which can either fix atmospheric nitrogen, solubilize phosphate, synthesis of growth promoting substances or by enhancing the decomposition of plant residues to release vital nutrients and increase humic content of soils, will beenvironmentally begin approach for nutrient management and ecosystem function [3].

EM technology is a scientifically unconfirmed method to improve soil quality and plant growth using a mixture of microorganisms consisting mainly of lactic acid bacteria, purple bacteria, and yeasts which co-exist for the benefit of whichever environment they are introduced. Now a days, exploitation of EM technology to improve the yield of various has been extensively increased in the various parts of the world [4,5,6].In the present study,plant growth promoting effect of effective microorganism like microbial consortia on *Arachis hypogea* was studied.

MATERIALS AND METHODS

Construction of Effective microorganism (EM) like microbial consortia

EM like microbial consortia was constructed from soil adopting soil dilution method]7].Soil sample was collected from paddy field in sterile polythene pack, Kept in air tight box and brought to the lab for further processing. Collected soil sample was sieved through nylon mesh and the sieved material was used for isolation studies.About 20 grams of sieved soil sample was suspended in 990 ml of sterile distilled water. 1 ml of the suspension was added into 99 ml of sterile distilled water blank, mixed well.1 ml of this alequate was further diluted. 0.1 ml of this alequate was spread plated on nutrient agar media, sabouraud dextrose agar media and starch casein agar media supplemented with antibiotics. The seeded plates were incubated at respective temperature (Bacteria and Actinomycetes – 37^{o}C and fungi – 28^{o}C). After the respective incubation period, microbial colonies were counted; pure culture was made and maintained on respective media agar slants at 4^{o}C. Identification of bacteria and actinomycetes was carried out by morphological, cultural and biochemical characteristics[8,9] fungi including yeast [10,11].

Determination of EM like microbial consortia microbial growth

To determine the growth rate or to study steady state growth rate of isolated microbial consortia or EM, sugarcane waste liquor media and minimal media was selected.(Minimal media was prepared with the following composition Sodium phosphate penta hydrate - 6.4 g,Potassium hydrogen phosphate - 1.5 g,Sodium Chloride - 0.25 g,Ammonium Chloride - 0.5 g,1 M Magnesium Sulphate solution - 0.2 ml,1 M Calcium Chloride - 0.1 ml,20% of glucose - 2 ml,Distilled Water - 100 ml).

Preparation of sugarcane waste liquor media

Sugarcane wastes were collected from sugarcane shop, kept in sterile polythene pack and soaked overnight in sterile distilled water. Soaked material was filtered through cheese muslin cloth. The collected filtrate was sterilized by autoclaving. After sterilization, the isolated respective bacterial, fungal, actinomycetes was inoculated and kept under shaking condition at 37^{o}C. Every 30 minutes, alequate of media was withdrawn and optical density was measured at 560nm.

Bacteria, yeast and actinomycetes were selected for the construction of EM like microbial consortia. They are *Lactobacillus plantarum , Flavobacterium flavus,Pseudomonas fluorescens,Streptomyces species,Saccharomyces cerevesiae,Candida utilis* and *Candida lipolytica*

Preparation of microbial consortia as EM stock

20 ml of minimal media was prepared in brown colour stoppered bottle (50 ml), sterilized by autoclaving and respective microbial pure culture was inoculated. The inoculated bottle was kept in room temperature for 24 hours. Changes in pH and development of odour (pleasant) was monitored or evaluated periodically.

Commercial formulation

Commercial formulation of EM-1 consisted of *Lactobacillus plantarum,Candida utilis, Streptomyces albus Aspergillus oryzae* was used in this study.

Application of EM

Description of the microplot

The microplot with the size of 5 × 3 meter leaving a gangway of one meter all around the pot (3) has been maintained for this study. The groundnut cultivar TMV-7 was sown during second week of November 2009 at a spacing of 30 × 10 cm and regularly water was sprayed without conventional agriculture practices, chemical synthetic fertilizers and pesticides were not applied. After 20 days after seeding emergence, the application was initiated with ultra low volume sprayer during evening time at less wind velocity. Control plot was sprayed with water only. Three replications were maintained for standard and test EM formulation. After treatment, the shoot length (cm), height of the plant (cm), leaf surface area (cm), total new branches emerged, total foliage density estimation, pest infestation, development of diseased spots, phyllosphere microbial population, total heterotrophic microbial population in soil, chlorophyll estimation, soil nitrogen, phosphorous and potassium level and cost benefit ratio was carried out every 15 days after treatment.

Measurement of shoot length and leaf surface area

After the EM application, the plant growth was measured by determining the length of the shoot, diameter of leaf surface area and foliage density. The shoot length and leaf surface area was measured randomly using measuring scale and total count of foliage density estimation was determined. This was done by flagging each branch. For plants with more than 100 leaves, each branch was tagged after all leaves of the branch were counted to avoid repetition.

Determination of pest infestation and diseased leaves

The effect of EM like microbial consortia on pest infestation and development of disease was determined by leaf (foliage damaged) and diseased spots or necrotic lesions[11].

Chlorophyll estimation

About 1 gram of leaves was homogenized and the homogenate was centrifuged at 5000Xg for 10 minutes. The pellet was washed twice with distilled water and suspend in 4 ml of methanol and vortexed thoroughly. Then the tube was incubated in water bath at 60^{o}C for one hour (in dark) with occasional shaking. After cooling, the tube was centrifuged at 7000 rpm for 5 minutes. The supernatant was transferred to another tube and once again 4 ml of the solvent was added and extracted again like above. To ensure complete extraction, once again 2 ml of solvent was added to the pellet and repeated the process. The supernatant was pooled made up to 10 ml with methanol (to compensate the solvent loss during heating). Optical density was read at 663nm against methanol blank. The amount of chlorophyll was calculated.

Determination of total heterotrophic microbial population from phyllosphere

Leaves from top, bottom and middle of the plants were collected after every 15 days interval till harvesting (80 days) in sterile polythene bags, kept in ice box and brought to the laboratory for microbiological analysis. The collected leaves (1 gram) were cut into small pieces (1 cm square surface), transfer to 100 ml of sterile distilled water, kept under shaking for 1 hour. 10 ml of the suspension was added into 90 ml of sterile

distilled water, and mixed well. One ml of this suspension was added into 9 ml of sterile distilled water and further serially diluted. 0.1 ml of the aliquate was spread plated on nutrient agar plates, sabourad dextrose agar plates, and starch casein agar plates. The plates were incubated at respective temperature and incubation period. After the incubation time, the respective microbial colonies were counted, purified and stored on respective agar slants. Respective microbial colonies were identified based on cultural characteristics.

Isolation of microorganisms from EM treated plot soil

The soil samples were periodically analysed for microbial analysis. The soil sample from respective treatment was analysed. Soil serial dilution method was used to determine soil microbial population. The isolated respective microbial colonies were identified based on cultural and biochemical characteristics.

Evaluation of soil nitrogen, phosphorous and potassium analysis

The soil samples were analysed for nitrogen, phosphorous and potassium content after one month of application. The analysis was carried out in Chennai Mettex laboratory, Guindy, Chennai.

Cost benefit ratio

The pod yield and number of healthy pods in respective treatment was also determined. Cost benefit ratio was calculated by using the following formula (Kalyanasundaram *et al* [12].

$$\text{Cost Benefit Ratio} = \frac{\text{Total gain}}{\text{Total cost of cultivation}}$$

Weather data

Temperature, mean relative humidity and rainfall were recorded from the nearest weather station. (Regional Meteorological Centre [RMC], Chennai) during the experimental period.

RESULT AND DISCUSSION

Effect of EM like microbial consortia on plant growth parameters

Groundnut (*Arachis hypagoea,* L.) (Fam.: Leguminaceae) is an important oil seed crop in India and the edible oil economy of the country primarily depends upon the groundnut production.Due to the high usage of synthetic chemicals as fertilizers,herbicides and pesticides ,production of groundnut is found to decline every year. The production of organic food requests for methods that use non-chemical inputs which is being extensively utilized for various crops. Input of bio principles in agricultures.In the present study,construction of microbial consortia like EM formulation was developed and their plant growth promoting effecting was studied against groundnut.

EM like microbial consortia inoculated groundnut plants recorded distinct plant growth parameters. These was a significant differences ($P \leq 0.05$) in shoot length, total height of the plant, leaf number per plant, leaf surface area, total new branch emerged, total foliage count per plant (Table 1).The length of shoot was found to be increased in all the tested time period in EM like microbial consortia (in test treatment) 28.0, 32.0, 37.5, 38.0 cm was recorded during 15^{th}, 30^{th}, 45^{th}, and 60^{th} days. In standard EM 29.0, 33.0, 35.0, 36.0 cm was recorded at respective time but in control, the length of shoot at respective time period was 24.0, 25.0, 26.5, 27.0 cm. The leaf surface area in test was found to be increased from 5.0 to 6.2, 6.1 in standard and 5.7 in untreated control. The total height of the plant was also increased in test than standard and control. The height of the plant at respective days was 31.0, 34.0, 39.0, 40.5 cm in test, 32.0, 37.0, 38.0, and 39.0 cm in standard and 26.0, 27.0, 28.0 and 30.0 cm in control.Total emergence of new branches was also increased in test. The number of new branches emerged per plant was found to be increased as 2.0, 3.0, 5.0, and 6.0 per plant during 15^{th}, 30^{th}, 45^{th}, and 60^{th} days after application in test. In standard, 2.0, 3.0, 4.0, and 5.0 new branches per plant were emerged at respective days. In control, the new branches emerged per plant was 2.0,

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2.0, 3.0, and 3.0 at respective days (table). Total foliage count per plant was also show distinct differences. In test treatment, the total foliage count per plant was 29.0, 42.0, 77.0, and 95.0, In standard 30.3, 40.1, 74.0, and 81.2, In control 31.0, 35.0, 49.0, and 52.0 per plant was recorded.

Table 1: Effect of EM like microbial consortia on growth parameters of groundnut plants

S.No	Treatment	Plant Growth Parameters	Before Treatment	Days After Treatment			
				15th	30th	45th	60th
1.	Control	Length of the shoot (cm)	22.0	24.0	25.5	26.5	27.0
2.	Standard		24.0	29.0	33.0	35.0	36.0*
3.	Test		23.0	28.0	32.0	37.5	38.0*
4.	Control	Leaf surface area (cm)	5.1	5.3	5.4	5.6	5.7
5.	Standard		5.0	5.3	5.6	5.9*	6.1*
6.	Test		5.0	5.4	5.6	5.9*	6.2*
7.	Control	Height of the plant (cm)	24.0	26.0	27.0	28.0	30.0
8.	Standard		26.5	32.0	37.0	38.0	39.0*
9.	Test		25.0	31.0	34.0	39*	40.5*
10.	Control	Total new branches emerged / plant	1.0	2.0	2.0	3.0	3.0
11.	Standard		1.0	2.0	3.0	4.0	5.0
12.	Test		1.0	2.0	3.0	5.0	6.0*
13.	Control	Total foliage count / plant	15.0	31.0	35.0	49.0	52.0
14.	Standard		14.2	30.3	40.1	74.0	81.2
15.	Test		15.0	29.0	42.0	77.0*	95.0*

***** Significant at P > 0.05 level by DMRT

Effect of EM like microbial consortia on pest infestation and diseased spots

There was distinct effect on pest infestation and disease spots developed was observed in EM like microbial consortia treated plots. The pest infestation per plant was 1.0, 0.8, 0.6, 0.5 and 0.5 in test. Similar observation was also recorded in standard. In control the number of pest infestation per plant was 0.3, 0.5, 0.62, 1.0 and 1.25. Similarly the disease spot appeared per plant was found to be reduced from 0.43, 0.41, 0.37, 0.35, 0.32, but in standard disease spotted leaf was increased from 0.3 to 0.37, 0.42, 0.54 and 0.68 per plant at respective time period. In untreated control 1.62, 2.1, 2.6, 3.0, 3.37 disease spots were appeared (Table 2)

Table 2: Effect of EM like microbial consortia on pest infestation/plant, disease spots/plant and chlorophyll content(mg/g)

S.No	Treatment	Parameters	Before Treatment	Days After Treatment			
				15th	30th	45th	60th
1.	Control	Pest infestation per plant	0.37	0.5	0.62	1.0	1.25
2.	Standard		1.0	0.8	0.62	0.54*	0.53*
3.	Test		1.0	0.8	0.6*	0.5*	0.5*
4.	Control	Disease spots per plant	1.62	2.1	2.6	3.0	3.37
5.	Standard		0.3	0.37	0.42	0.54	0.68
6.	Test		0.7	0.6	0.4*	0.3*	0.25*
7.	Control	Chlorophyll estimation (mg/g)	0.32	0.35	0.37	0.41	0.43
8.	Standard		0.32	0.35	0.37	0.41	0.43
9.	Test		0.32	0.35	0.37	0.41	0.43

* Significant at P > 0.05 level by DMRT

Effect of EM like microbial consortia on chlorophyll content

There was no distinct differences was observed in chlorophyll content in test, standard and control. 0.32, 0.35, 0.37, 0.41 and 0.43 (mg/g) was recorded in control, standard and EM like microbial consortia respectively.

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Effect of EM like microbial consortia on phyllosphere population

As in plant growth parameters, microbial population of phyllosphere was found to be increased in test EM like microbial consortia treated plot 28.6×10^5, 34.3×10^5, 36.9×10^5, 38.6×10^5 CFU/g bacteria, yeast and mold 17.7×10^5, 25.8×10^5, 28.7×10^5, 29.3×10^5 CFU/g and actinomycetes 8.9×10^5, 11.3×10^5, 14.6×10^5, 14.8×10^5 CFU/g was recorded but the bacteria, yeast and mold and actinomycetes population before treatment was 20.9×10^5, 14.8×10^5, 5.3×10^5 CFU/g.In standard, 24.3×10^5, 25.7×10^5, 28.6×10^5, 31.1×10^5 CFU/g bacteria, yeast and mold 8.7×10^5, 11.2×10^5, 12.2×10^5, 14.5×10^5 CFU/g and actinomycetes 7.4×10^5, 8.7×10^5, 9.3×10^5, 10.6×10^5CFU/g was recorded but the bacteria, yeast and mold and actinomycetes population before treatment was 21.2×10^5, 6.9×10^5, 6.6×10^5 CFU/g.In control, 21.1×10^5, 23.0×10^5, 24.4×10^5, 25.6×10^5CFU/g bacteria, yeast and mold 9.6×10^5, 11.0×10^5, 12.9×10^5, 15.3×10^5CFU/g and actinomycetes 9.1×10^5, 11.5×10^5, 13.2×10^5, 14.7×10^5 CFU/g was recorded but the bacteria, yeast and mold and actinomycetes population before treatment was 17.5×10^5, 6.8×10^5, 7.5×10^5 CFU/g (Table 3).

Table 3: Effect of EM like microbial consortia on microbial population of groundnut plants

S.No	Treatment	Microbial population	Before Treatment	Days After Treatment			
				15th	30th	45th	60th
1.	Control	Microbial population of phyllosphere	17.5×10^5	21.1×10^5	23.0×10^5	24.4×10^5	25.6×10^5
2.	Standard	Bacteria	21.2×10^5	24.3×10^5	25.7×10^5	28.6×10^5	31.1×10^5
3.	Test		20.9×10^5	28.6×10^5	34.3×10^5	36.9×10^5	38.6×10^5
4.	Control	Microbial population of phyllosphere	6.8×10^5	9.6×10^5	11.0×10^5	12.9×10^5	15.3×10^5
5.	Standard	Yeast and Mold	6.9×10^5	8.7×10^5	11.2×10^5	12.2×10^5	14.5×10^5
6.	Test		14.8×10^5	17.7×10^5	25.8×10^5	28.7×10^5	29.3×10^5
7.	Control	Microbial population of phyllosphere	7.5×10^5	9.1×10^5	11.5×10^5	13.2×10^5	14.7×10^5
8.	Standard	Actinomycetes	6.6×10^5	7.4×10^5	8.7×10^5	9.3×10^5	10.6×10^5
9.	Test		5.3×10^5	8.9×10^5	11.3×10^5	14.6×10^5	14.8×10^5
10.	Control	Microbial population of soil	20.6×10^5	21.0×10^5	23.6×10^5	27.3×10^5	28.6×10^5
11.	Standard	Bacteria	23.4×10^5	28.6×10^5	33.2×10^5	35.6×10^5	39.2×10^5
12.	Test		24.3×10^5	39.6×10^5	48.6×10^5	50.8×10^5	54.4×10^5
13.	Control	Microbial population of soil	13.4×10^5	14.3×10^5	16.6×10^5	19.2×10^5	21.9×10^5
14.	Standard	Yeast and mold	6.1×10^5	6.3×10^5	7.1×10^5	8.9×10^5	9.8×10^5
15.	Test		4.1×10^5	4.3×10^5	5.8×10^5	8.2×10^5	9.5×10^5
16.	Control	Microbial population of soil	7.1×10^5	7.3×10^5	8.7×10^5	9.6×10^5	10.3×10^5
17.	Standard	Actinomycetes	4.7×10^5	5.9×10^5	7.7×10^5	8.5×10^5	10.7×10^5
18.	Test		8.7×10^5	12.1×10^5	14.2×10^5	19.7×10^5	23.4×10^5

Effect of EM like microbial consortia on soil population

As in plant growth parameters microbial population of soil was found to be increased in test EM like microbial consortia treated plot (Table 3). 39.6×10^5, 48.6×10^5, 50.8×10^5, 54.4×10^5 CFU/g bacteria, yeast and mold 4.3×10^5, 5.8×10^5, 8.2×10^5, 9.5×10^5 CFU/g and actinomycetes12.1×10^5, 14.2×10^5, 19.7×10^5, 23.4×10^5 CFU/g was recorded but the bacteria, yeast and mold and actinomycetes population before treatment was 24.3×10^5, 4.1×10^5, 8.7×10^5 CFU/g.In standard, 28.6×10^5, 33.2×10^5, 35.6×10^5, 39.2×10^5 CFU/g bacteria, yeast and mold 6.3×10^5, 7.1×10^5, 8.9×10^5, 9.8×10^5 CFU/g and actinomycetes 5.9×10^5, 7.7×10^5, 8.5×10^5, 10.7×10^5 CFU/g was recorded but the bacteria, yeast and mold and actinomycetes population before treatment was 23.4×10^5, 6.1×10^5, 4.7×10^5 CFU/g.In control, 21.0×10^5, 23.6×10^5, 27.3×10^5, 28.6×10^5 CFU/g bacteria, yeast and mold 14.3×10^5, 16.6×10^5, 19.2×10^5, 21.9×10^5 CFU/g, and actinomycetes 7.3×10^5, 8.7×10^5, 9.6×10^5, 10.3×10^5 CFU/g was recorded but the bacteria, yeast and mold and actinomycetes population before treatment was 20.6×10^5, 13.4×10^5, 7.1×10^5 CFU/g.

Effect of EM like microbial consortia on total nitrogen, phosphorous and potassium content

EM like microbial consortia treated soil showed drastic increase in total nitrogen, phosphorous and potassium than standard and control. 21.0, 16.87, 71.42 mg/kg of nitrogen, phosphorous and potassium was recorded in test treatment, 9.8, 41.62 and 40.0 mg/kg in standard, 14.0, 6.76, 37.53 mg/kg in untreated control was recorded (table 4).

Table 4: Effect of EM like microbial consortia on total nitrogen, phosphorous and potassium[pi8i8 86 content in soil

S.No	Treatment	Before Treatment			After Treatment		
		Nitrogen (mg/kg) Alkali $KMnO_4$	Phosphorous (mg/kg) Olsen's method	Potassium (mg/kg) Flame Photometric	Nitrogen (mg/kg) Alkali $KMnO_4$	Phosphorous (mg/kg) Olsen's method	Potassium (mg/kg) Flame Photometric
1.	Control	7.0	1.4	34.0	14.0	6.76	37.53
2.	Standard	7.0	1.3	35.4	9.8	41.62	40.0
3.	Test	7.1	1.2	35.1	21.0	16.87	71.42

Effect of EM like microbial consortia on pod yield and cost benefit ratio

Pod yield per plant was also increased in test EM like microbial consortia applied plots than standard and control 18.0 pods per plant and 16.0 healthy pods per plant was obtained (Table 5). In standard EM treated plots pod yield 12.0 and number of healthy pods 8.0. In untreated control pod yield only 8.0 and number of healthy pods 3.0. The cost benefit ratio was also found to be high in tested EM like microbial consortia plot (1:1.93) followed by standard EM 1:1.86 and control recorded in control plot was 1:1.09) Total yield was high in EM like microbial consortia treated plot..Intersetingly,climatic condition did not affect the parameters(Table 6).The present study clearly reveals the possible utilization of EM like microbial consortia as the plant growth promoter and further study will help to exploit the principles of EM like consortia as the organic fertilizer.

Table 5: Total yield and cost benefit ratio of EM like microbial consortia treated groundnut

S.No.	Treatment	Total yield	Cost benefit ratio
1	Control	641.5	1:1.09
2	Commercial formulated EM	907.2	1:1.86
3	EM like microbial consortia	1021.3	1:1.93

Table 6: Weather data recorded during the study period

Month	Days	Standard week	Temperature (^oC)		Rainfall (mm)	Relative Humidity (%)
			Maximum	Minimum		
November	15 - 22	1	24.12	21.17	17.21	86.1
	23 - 30	2	24.17	21.19	11.13	92.3
December	1 - 8	3	22.12	19.17	6.17	91.5
	9 - 16	4	22.17	19.21	-	95.6
	17 - 24	5	24.34	21.82	4.12	93.8
	25 - 31	6	23.71	21.17	-	84.1
January	1 - 8	7	24.12	21.34	-	60.3
	9 - 16	8	23.12	21.11	3.17	65.2
	17 - 24	9	26.11	22.17	-	63.9
	25 - 31	10	25.12	22.17	-	56.4
February	1 - 8	11	26.17	23.12	-	58.7
	9 - 16	12	24.17	21.19	-	60.6
	17 - 24	13	26.19	23.14	-	56.5

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